This report is required by law (7 USC 2143). Failure to report according to the regulations can result in an order to cease and desist and to be subject to penalties as provided for in Section 2150.

See reverse side for additional information.

Interagency Report Control No 0180-DOA-AN

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

ANNUAL REPORT OF RESEARCH FACILITY

(TYPE OR PRINT)

1. REGISTRATION NO. 10-F-0002 **CUSTOMER NO.** 439

FORM APPROVED
OMB NO. 0579-0036

2. HEADQUARTERS RESEARCH FACILITY *(Name and Address, as registered with USDA, include Zip Code)*

Walter Reed Army Institute of Research/Naval Medical Research Center
Division of Veterinary Medicine
Building. 511 Robert Grant Avenue
Silver Spring, MD 20910-7500
Telephone (301) 319-9811

3. REPORTING FACILITY (List all locations where animals were housed or used in actual research, testing, teaching, or experimentation, or held for these purposes. Attach additional sheets if necessary.)

FACILITY LOCATIONS *(sites)*

See Attached Listing

(b)(2)High, (b)(7)f

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY *(Attach additional sheets if necessary or use APHIS FORM 7023A)*

A. Animals Covered By The Animal Welfare Regulations	B. Number of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes.	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.	E. Number of animals upon which teaching, experiments, research, surgery or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic,analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. *(An explanation of the procedures producing pain or distress in these animals and the reasons such drugs were not used must be attached to this report)*	F. TOTAL NO. OF ANIMALS (Cols. C + D + E)
4. Dogs					
5. Cats					
6. Guinea Pigs	0	182	200	172	554
7. Hamsters	0	0	68	0	68
8. Rabbits	0	93	46	0	139
9. Non-Human Primates	208	177	11	15	203
10. Sheep	0	0	3	17	20
11. Pigs	0	4	468	2	474
12. Other Farm Animals					
13. Other Animals					
14. Ferrets	0	0	60	7	67
15. Sand Rats	0	0	30	0	30

ASSURANCE STATEMENTS

1) Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anesthetic, analgesic, and tranquilizing drugs, prior to, during, and following actual research, teaching, testing, surgery, or experimentation were followed by this research facility.

2) Each principal investigator has considered alternatives to painful procedures.

3) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and approved by the Institutional Animal Care and Use Committee (IACUC). **A summary of all the exceptions is attached to this annual report.** In addition to identifying the IACUC-approved exceptions, this summary includes a brief explanation of the exceptions, as well as the species and number of animals affected.

4) The attending veterinarian for this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL
(Chief Executive Officer or Legally Responsible Institutional official)
I certify that the above is true, correct, and complete (7 U.S.C. Section 2143)

(b)(6), (b)(7)c

DATE SIGNED
19 Nov 07

1 - HEADQUARTERS

Column E Explanation

This form is intended as an aid to completing the Column E explanation. It is not an official form and its use is voluntary. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

1. Registration Number: 10-F-0002

2. Number of animals used in this study 172.

3. Species of animals used in the study (common name) Guinea Pig.

4. Explain the procedure producing pain and/or distress.

Shigella vaccine candidates were evaluated by placing Shigella in the conjuctivae of guinea pigs' eyes, and then the severity of inflammation was scored.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below)

The study of immune response to and protective efficacy of vaccine candidates directed against Shigella requires an accurate evaluation of the immune response raised by the administration of these vaccines. The use of analgesics, particularly opiates or narcotics, result in immunosuppression, which would invalidate the results of experiments testing immune responses as well as increasing the severity of the possible eye infection. Use of analgesics that are anti-inflammatory (e.g. aspirin) would also invalidate the model since we are studying a model for inflammation of epithelial cells by bacterial invasion.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

Agency None CFR _____

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1. Registration Number: 10-F-0002

2. Numberof animals used in this study: 15.

3. Species (common name) of animals used in the study: Rhesus Macaques.

4. Explain the procedure producing pain and/or distress.

Animals will be challenged via aerosol administration with *Francisella tularensis LVS* strain SCHU-S4 9 weeks after test vaccine administration.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below)

The aerosol exposure of virulent tularemia is critical to assessing the relative efficacy of the different routes of inoculation of the LVS vaccine to protect against aerosol exposure. Because the measurement of efficacy in tularemia is dependent upon a lethal model it is important that the disease process be interfered with as little as possible. Because of the potential for analgesics to contribute to or exacerbate the symptoms of shock; it is unwise to use them in this protocol. Because fever is a major statistical parameter being used to evaluate the animal response to both the vaccine and challenge, non-steroidal anti-inflammatory drugs (NSAIDs) will not be used in this protocol.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

Agency None CFR _____

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1. Registration Number: 10-F-0002

2. Number of animals used in this study 17.

3. Species of animals used in the study (common name) Sheep.

4. Explain the procedure producing pain and/or distress.

Experiments will examine the consequential effects of inhalation exposure to CO or NO_2 (singly or in combination) on sheep physiology and biochemistry. Attention will be given to those factors that may contribute to the amount of toxic gas that is taken up in the body during normal and simulated mouth breathing (dose determinants). In addition, a non-invasive biomarker of pulmonary inflammation will be investigated. Some substernal stress and bronchoconstriction may be experienced in sheep.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below)

The only unalleviated pain, stress, or distress that may occur, will occur as a direct result of NO_2 exposure, particularly occurring on a delayed basis (approaching 24-h post exposure). However, study endpoints include neither death nor near-death. Rather a more subtle physio-chemical endpoint (hypoxemia) is sought. Some substernal stress and bronchoconstriction may be experienced in a sheep exposed to the highest gas exposure equivalent. This experience may be better classified as "stress and discomfort", rather than pain (a bronchospastic response is more stressful than painful). However, therapeutic corrective measures for these few animals (*ie*, bronchodilators, hyperoxia, analgesics that coincidentally alter cardiopulmonary function, *etc*) could directly or indirectly mask the desired endpoint. The PI has consulted with the attending veterinarian or his or her designee in the planning of both alleviated and unalleviated painful procedures.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

Agency None CFR _____

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1. Registration Number: 10-F-0002

2. Number of animals used in this study 2.

3. Species of animals used in the study (common name) Swine.

4. Explain the procedure producing pain and/or distress.

Piglets will be exposed to Staphylococcal enterotoxins and bacterial lipopolysaccharide (LPS), which in some animals is expected to lead to lethal toxic shock. This study will be extended to examine similar responses induced in piglets by a shock causing bacteria and virus. Mediators in expired breath will be measured and correlated with associated pathogen-specific disease pathology and illness progression.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below)

The goal of this protocol is to correlate the kinetics of: a) Gene expression changes (increases or decreases in select genes); b) Protein profiles (proteins in sera / Cytokines / antibodies) and c) Other early mediators (cytokines / leukotrienes) from breath and blood or tissues with physical symptomology and physiology of the piglets exposed to either Staphylococcal enterotoxins toxins and/or bacterial and viral pathogens. Hence, addition of any pain alleviators or analgesics will change the gene expression profiles and protein profiles in the infected piglets. It is already known that analgesics or anesthetics can mask protein profiles from breath and even alter gene levels.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

Agency None CFR _____

Column E Explanation

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1. Registration Number: 10-F-0002

2. Number of animals used in this study 7.

3. Species of animals used in the study (common name) Ferrets.

4. Explain the procedure producing pain and/or distress.

Ferrets will receive vaccine or control material then challenged with *Campylobacter* bacteria to see if the animals develop strong enough immunity to resist the infection. Ferrets may experience the discomfort of diarrhea and possible, but unlikely dehydration.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below)

Approximately 10% of the animals are placed in pain category E. These include animals that may have >2+ *Campylobacter*-associated diarrhea. These animals cannot be treated with analgesics or antibiotics, because the results obtained will be confounding and the recovery or no sickness of immunized animals cannot be attributed to the efficacy of the vaccine. Analgesics are contraindicated since these agents would change the course of the disease and alter physiologic/pathologic endpoints. Therefore, no such pharmacological agents will be used due to the risk of interference with the outcome of the disease.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

Agency None CFR _____